BIO-TECHNOLOGY

Time allowed : 3 hours ***Maximum marks: 70***

General Instructions:

(i) ***All*** *questions are compulsory.*

(ii) *There is no overall choice. However, an internal choice has been provided in one question of two marks and two questions of five marks. You have to attempt only one of the choices in such questions. Question paper contains four sections -A, B, C and D.*

(iii) *Question numbers* ***1*** *to* ***5*** *are very short answer questions, carrying* ***1*** *mark each.*

(iv) *Question numbers* ***6*** *to* ***15*** *are short answer questions, carrying* ***2*** *marks each.*

(v) *Question numbers* ***16*** *to* ***25*** *are also short answer questions, carrying* ***3*** *marks each.*

(vi) *Question numbers* ***26*** *to* ***28*** *are long answer questions, carrying* ***5*** *marks each.*

(vii) *Use of calculators is not permitted. However, you may use log tables, if necessary.*

QUESTION PAPER CODE 99/1

Section - A

1. Give the sequence of the 2 primers (5-nucleotides long) required to amplify the following DNA sequence by PCR :

5'ATGCCTAGGATCATGC 3' 1

2. Why is the nutrient medium autoclaved before using it for culturing microbes ? 1

3. Which future vaccine holds promise of bypassing the need to visit the doctor regularly for childhood immunisations ? 1

4. A soil microorganism produces a novel metabolite in nanomolar concentration (nM). Suggest a way to increase its production in quantities that are economically viable. 1

5. Why is 'Golden rice' nutritionally superior to normal rice ? 1

Section - B

6. Ovalbumin is the major protein of egg white. The chicken ovalbumin gene contains 8 exons separated by 7 introns. Should one use ovalbumin cDNA or genomic DNA to express the protein in *E. coli* and why ? 2

7. How can SNPs be used to predict susceptibility to diseases ? 2

8. What is the mode of action of tissue plasminogen activator (t-PA) ? Name one medical application of t-PA. 2

9. Which one of the following proteins would be expected to migrate fastest through SDS-PAGE gel and why ?

Protein	MW (daltons)
α –macroglobulin	820,000
Lysozyme	15,000
Serum albumin	69,000
Retinol binding protein	21,000
α –antitrypsin	45,000

2

10. Why are type II restriction endonucleases (RE) extensively used in recombinant DNA technology ? Why do bacteria make RE ? 2

11. What is the IUPAC code for T or C ? Write the complementary sequence of the following sequence :

5'-ATGAYCGBT -3' 2

12. Why is foaming caused in microbiological processes ? Name a commonly used anti-foaming agent. 2

13. Erythropoietin (EPO) is included in the list of banned substances for sportsmen. What is this substance ? How does it act ? 2

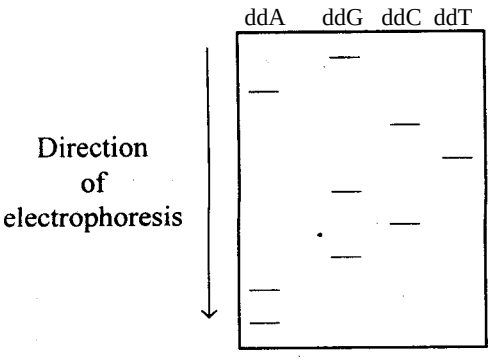
OR

Embryonic cells during development not only commit along different lineages but also retain a population of cells that are present only at strategic locations in the adult organism. Name these specialized cells and why they are maintained in undifferentiated state? 2

14. An autoradiogram of a sequencing gel containing 4 lanes of DNA fragments is shown in the figure below :

(i) Read the DNA sequence from the autoradiogram.

(ii) What purpose do ddNTPs serve in Sanger's method of DNA sequencing ? 2



15. Study the following enzyme purification table and answer the questions that follow:

Step	Procedure	Total protein (mg)	Activity (units)
1.	Crude extract	20,000	4,000,000
2.	Precipitation (salt)	5,000	3,000,000
3.	Precipitation (pH)	4,000	1,000,000
4.	Ion exchange chromatography	200	800,000

(a) Which step in the purification is most effective, and why ?

(b) Which of the procedures is least effective and why ? 2

Section-C

16. Why is the technique for the production of monoclonal antibodies called hybridoma technology. ? Why are monoclonal antibodies preferred over serum antibodies in diagnostics and therapeutics ? Give an example of a therapeutic use of monoclonal antibody. 3

17. What is 'Molecular Pharming' ? Suggest any four advantages of expressing transgenic proteins in milk. 3

18. Suggest any four reasons why complete genome sequencing projects should be undertaken ? Describe the advantage of using bacterial artificial chromosomes (BAC) in such sequencing programmes. 3

19. What is downstream processing ? What strategy would you use to purify a recombinant protein that is secreted into the growth medium ? 3

20. What are the basic steps of a polymerase chain reaction (PCR) ? Give two applications of PCR. 3

21. How can you obtain virus-free sugarcane plants from virus-infected plants ? Are these plants virus resistant ? Why or why not ? 3

22. Why is it difficult to culture animal cells as compared to microbial cells ? How is the pH and osmolality of the medium monitored and maintained in animal cell culturing ? 3

23. Name any four physical and/or chemical properties of enzymes which might be useful to change by site-directed mutagenesis. Support your answer by taking an example of an engineered protein/enzyme. 3

24. Explain how DNA "microarray" technique can be used to study cellular response to environment. Also depict major steps diagrammatically. 3

25. What are microbial culture collection centres ? Suggest any two benefits. Name any one culture collection centre and its location. 3

Section - D

26. (a) What is the principle of protein finger printing ? Illustrate major steps.
(b) Who developed this technique ?
(c) Name a human disease caused by the absence of a protein /enzyme. 5

27. (a) Enlist the four major steps in a recombinant DNA experiment.
(b) What is the advantage of having a poly linker in a cloning vector ?
(c) Name a cloning vector that can be used to clone large DNA fragments (> 1 MB) 5

OR

(a) What is the principle of blue-white selection for the identification of recombinants ?
(b) Name any three methods of introducing recombinant DNA into host cells. 5

28. (a) Enlist the six major steps in plant tissue culture.
(b) Name a medium commonly used for culturing plant parts and what factors dictate the choice of media ? 5

OR

(a) Describe vector-mediated and vector-less gene transfer in plants.
(b) Why is *Agrobacterium tumefaciens* regarded as nature's genetic engineer ? 5

QUESTION PAPER CODE 99

SECTION - A

1. Give the sequence of the two primers (5 nucleotides long) required to amplify the following DNA sequence by PCR : 1

5' GCACCTAGATCGATCC 3'

2. What is lyophilization ?

3. What treatment will you recommend to a fruit-seller for ripening a consignment of 'Flavr Savr' tomatoes, and why ? 1

4. A soil micro-organism produces a novel metabolite in nanomolar concentration (nM). Suggest a way to increase its production in quantities that are economically viable. 1

5. Suppose you are planning a large scale hybridisation programme in maize, how can this task be made less labour intensive ? 1

SECTION - B

6. You wish to introduce the human insulin gene into a bacterial host in the hope of producing large amounts of human insulin. Should you use genomic DNA or cDNA ? Explain. 2

7. What are ESTs ? How are they useful in genome analysis ? 2

8. What is the mode of action of tissue plasminogen activator (t-PA) ? Suggest one medical application of t-PA. 2

9. Which of the following proteins would be expected to migrate fastest through SDS-PAGE gel, and why ? 2

Protein	*MW (daltons)*
Transferrin	90,000
Cytochrome c	13,400
α-antitrypsin	45,000
Myoglobin	17,000
Serum albumin	69,000

10. Give two distinguishing features of pBR322 and pUC19 vectors. 2

11. What is the IUPAC code for A or C ? Write the complementary sequence of the following sequence : 2

5'-ASGYTWCAG-3'

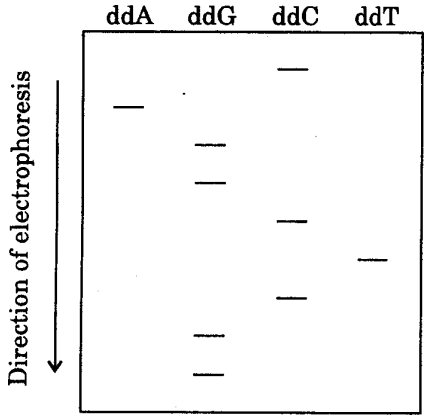
12. Why is aeration important for microbial growth ? How can proper aeration be achieved in microbial cultures grown under laboratory conditions ? 2

13. The long distance runners are disqualified if they test positive for erythropoietin (EPO). What is this substance and how does it act ? 2

OR

Embryonic cells during development not only commit along different lineages but also retain a population of cells that are present only at strategic locations in the adult organism. What are these specialized cells known as ? Why are they maintained in undifferentiated state ?

14. An autoradiogram of a sequencing gel containing 4 lanes of DNA fragments is shown in the figure below :



(a) Read the DNA sequence from the autoradiogram.

(b) Explain why the sequence read from the autoradiogram is complementary to the original sequence. 2

15. Study the following enzyme purification table and answer the questions that follow :

Step	Procedure	Total protein (mg)	Activity (units)
1.	Crude extract	15,000	1,50,000
2.	Salt fractionation	4,000	1,38,000
3.	Ion exchange chromatography	1,500	1,15,500
4.	Molecular exclusion chromatography	688	75,000
5.	Affinity chromatography	1-75	52,000

(a) Which step in the purification is most effective, and why ?

(b) Which of the procedures is least effective, and why ? 2

SECTION C

16. What is OKT-3 ? Why is it administered to patients undergoing organ transplantation ? What is the relevance of fusing an antibody producing B-cell with myeloma cells in hybridoma technology ? 3

17. What is 'Molecular Pharming' ? Suggest any four advantages of expressing transgenic proteins in milk ? 3

18. Name any three resources available from the NCBI and their uses. 3

19. What is fed-batch culture and what are its benefits in microbial technology ? How is it different from a batch culture ? 3

20. Name the special DNA polymerase used in PCR reactions. What are the three basic steps of a PCR cycle ? Using a single template molecule, how many DNA molecules are generated after 10 cycles of amplification ? 3

21. What are edible vaccines ? Give 3 advantages of developing edible vaccines. Which plant part(s) will be best suited for expressing antigenic transgene ? 3

22. Why is it difficult to culture animal cells as compared to plant or microbial cells ? How is the pH and osmolality of the medium monitored and maintained in animal cell culturing ? 3

23. Name any four physical and/or chemical properties of enzymes which might be useful to change by site-directed mutagenesis. Support your answer by taking an example of an engineered protein/enzyme. 3

24. A Chronic Myelogenous Leukemia (CML) patient has been put on a combination drug therapy for the past 2 months. How can the FISH technique be used to monitor the effect of chemotherapy ? 3

25. What are the microhial culture collection centres ? Suggest any two benefits. Name a microbial culture centre from India and its location. 3

SECTION D

26. (a) What is the principle of protein fingerprinting ? Illustrate major steps.

(b) Who developed this technique ?

(c) What are prions ? 5

27. (a) How will you select bacterial cells carrying a recombinant plasmid ?

(b) Explain briefly a technique for visual screening of transformed bacteria.

(c) How can *E. coli* cells be made competent and who developed this method ? 5

OR

(a) Enlist the four major steps in a recombinant DNA experiment.

(b) What is the advantage of having a polylinker in a cloning vector ?

(c) Name a cloning vector that can be used to clone large DNA fragments (> 1 MB).

28. (a) Describe vector-mediated and vector-less gene transfer in plants.

(b) Why is *Agrobacterium tumefaciens* regarded as nature's genetic engineer ? 5

OR

(a) Enlist the six major steps in plant tissue culture.

(b) Name a medium commonly used for culturing plant parts and what factors dictate the choice of media.

Marking Scheme — Biotechnology

QUESTION PAPER CODE 99/1

EXPECTED ANSWERS/VALUE POINTS

SECTION A

1.	Primer1 5' GCATG 3' [in any order] Primer2 5'ATGCC 3'	½×2=1
2.	To sterilize medium, which kills microbes	1
3.	Edible vaccines	1
4.	Strain improvement by inducing mutations followed by selection.	1
5.	Golden rice is a genetically engineered rice. It has genes for producing precursor of Vitamin A (carotenoids) in the grain. Hence, nutritionally superior.	1

SECTION B

6.	cDNA: *E coli* lacks the machinery to splice primary transcripts arising from genomic DNA.	1+1=2
7.	Genomic variations underlie differences in our susceptibility to diseases / Hence, by analyzing SNP, susceptibility to disease can be predicted.	1+1=2
8.	It dissolves blood clots / Administrated after a heart attack.	1+1 =2
9.	Lysozyme. Lower the MW, faster is the migration through the gel.	1+1 =2
10.	They recognize a sequence & cut within the sequence to produce reproducible fragments. They restrict the propagation of any foreign DNA in bacteria.	1 + 1=2
11.	Y, 3' TACTRGCVA5' or 5' AVCGRTCAT3'	1 + 1=2
12.	Foaming is due to proteins / components of the medium / metabolites produced by microbes. Olive oil etc.	1+1=2
13.	Erythropoietin is a hormone. It increases production of RBC leading to increased O_2 carrying capacity. This leads to increased aerobic metabolism and hence improved performance.	1+1=2
	OR	
	Stem cells can either divide to maintain their numbers or differentiate in response to need to renew the lost cells.	1+1=2
14.	5' AAG C G TCAG 3' / chain termination / No free 3' OH is available	1 + ½+½ = 2
15.	(a) STEP 4, Maximum specific activity (b) STEP 3, Least specific activity from the preceding step.	½×4 =2

SECTION C

16. For producing monoclonal antibodies, Abs producing B-cells are fused with myeloma cells (immortal) leading to hybridoma

    Monoclonal Abs are epitope specific whereas serum Abs are heterogenous.
    OKT-3 (anti CD3 Mo Ab) is effective in graft survival $1\times3 = 3$

17. It is the creation of transgenic animals expressing proteins in milk.

    Advantages:

    (i) High production capacity
    (ii) Ease of collection of source material
    (iii) Moderate capital instrument requirements and low operational cost.
    (iv) Ease of production, scale up. $1+ ½\times4 = 3$

18. **Reasons for sequencing :**

    (i) provides a basis for discovery and hence inventory of all the genes
    (ii) shows relationship between genes
    (iii) tools for future experimentation
    (iv) index to draw and organize all genetic information about the organism
    (v) provides an archive for the future (ANY FOUR)

    Advantages of BAC : BAC are capable of stably propagating large DNA inserts in *E.coli* $½\times4+1 = 3$

19. Recovery of desired metabolite from the cells with or without cell disruption is called downstream processing.

    Strategy for isolation of extracellular recombinant protein

    Fermentation $\rightarrow$ drum filter $\rightarrow$ clear broth $\rightarrow$ liquid extraction/chromatography, concentration $\rightarrow$ purification, crystallization $\rightarrow$ pure recombinant protein $1+2=3$

20. Denaturation, primer annealing & chain extension.

    Applications:

    (i) To detect pathogen specific sequences.
    (ii) DNA finger printing (or any other) $1 + 1+1=3$

21. Meristems from virus-infected plants are used for initiation of culture. This is followed by shoot induction, rooting & transplantation.

    They are not virus-resistant as they are derived from virus sensitive plants.

    $2+½ + ½ = 3$

22. (i) Animal cells have complex nutritional requirements
    (ii) require special incubators for maintaining the level of O_2/CO_2
    (iii) temperature
    (iv) humidity (ANY TWO)

Parameter	Monitored	Maintainance
pH	Indicators	Buffers
Osmolality	osmometer	osmolytes

½×6 =3

23. (i) Increased thermal stability
(ii) Increased resistance to denaturation by organic solvents
(iii) Increased resistance to extremes of pH
(iv) Improved catalytic efficiency
(v) Modify specificity *(ANY FOUR)*

Explain by taking the example of subtilisin where MET → ALA change has lead to increase in the stability of this protein ½×4 +1 = 3

24. First of all, mRNA is purified from the two types of cells → cDNA is made with incorporation of two different fluorescent dyes → hybridization with probes on the microarray followed by laser scanning.

Diagram from page 85 (Fig. 3) 1+2 = 3

25. Culture collection centre are places where microbial cultures can be deposited and maintained.

Benefits:

(i) Maintain culture for longer period
(ii) provide experimental material to Investigators
(iii) help in protecting intellectual property rights of depositors *(ANY TWO)*

Example: ATCC (USA)/DSM (Germany) /MTCC (India) 1+ ½×2 +1 = 3

Section D

26. (a) Principle: Each protein is identified by its unique peptide map

STEPS: purification → trypsin treatment→ paper electrophoresis → paper chromatography → peptide sequencing

(b) V. M. Ingram (1957)

(c) Thalassemia /SCID / any other 3+1+1=5

27. (a) Steps
(i) isolation of DNA fragments (inserts)
(ii) generation of rDNA (vector+insert)
(iii) introduction of rDNA into host cell
(iv) selection of the desired rDNA

(b) Polylinker/MCS has unique recognition sites for several REs. It provides flexibility on the choice of REs that.can be used for cloning.

(c) YAC 3+1½ + ½ = 5

OR

(a) Insertional inactivation of lacZ gene present in the vector
lacZ encodes for the enzyme β-galactosidase which can cleave X-gal into a blue colour product. If this gene is inactivated, blue colour is prevented.

(b) transformation, transfection, electroporation, biolistics, microinjection, phage *(ANY THREE)* 2+3=5

28. (a) Six steps:
(i) selection of explant
(ii) surface sterilization of explant
(iii) inoculation of explant on to suitable nutrient medium
(iv) growing the culture in the PTC room
(v) regeneration of plant
(vi) acclimatization

(b) Murashige & Skoog's medium (MS), type of plant species or the plant part. ½×6+1+1=5

OR

(a) Vector mediated: using *Agrobacterium* mediated transformation (details pp-127)

Vectorless: chemical mediated/microinjection/electroporation and biolistic

(b) It has the natural ability to transfer T-DNA of their plasmid into plant genome upon infection of cells at wound sites. 4+1 = 5

QUESTION PAPER CODE 99

EXPECTED ANSWERS/VALUE POINTS

SECTION A

1. Primer 5' GCACC 3' [in any order] ½×2 = 1
Primer 5' GGATC 3'

2. Lyophilisation involves freezing (e.g. of a culture) followed by drying under vacuum. 1

3. Application of ethylene; promotes fruit ripening. ½ +½ = 1

4. Strain improvement by inducing mutations followed by selection. 1

5. Use male sterile lines. Eliminates need for emasculation. 1

SECTION B

6. cDNA: *E coli* lacks the machinery to splice primary transcripts arising from genomic DNA. 1 + 1=2

7. ESTs expressed sequence tags are part of DNA sequences from expressed portions of the genome. Could be used as handles to isolate the entire gene or could be used to infer expression patterns (any one) ½+½+1 = 2

8. It dissolves blood clots. Administrated after a heart attack. 1+1=2

9. Cytochrome C.
   Lower the molecular weight, higher is the migration through the gel. 1+1=2

10. Consider any of the two points listed for each plasmid. pBR322- Amp and Tet resistant, no MCS, insert causes loss of one antibiotic resistance (insertional inactivation). For pUC19 - Amp resistance, has MCS for insert, has lac Z gene which can be inactivated by insert. ½×4 = 2

11. M, 3' TSCRAWGTCS5' or 5' CTGWARCST 3' 1 + 1=2

12. For efficient oxygen transfer, increased ATP production and mixing; using shakers and baffle flasks. 1+1=2

13. Erythropoietin is a hormone ; it increases production of RBC, increased O_2 carrying capacity, leading to increased aerobic metabolism and hence improved performance. 1+1=2

OR

Stem cells: can either divide to maintain their numbers or differentiate in response to renew the lost cells. 1+1=2

14. 5' GGCTCGGAC3' ; Sequencing techniques involves incorporation of complementary base pairs. 1 + 1=2

15. (a) STEP 5, Maximum specific activity
    (b) STEP 3, Least specific activity from the preceding step. $^1/_2$ x4 =2

SECTION C

16. It is a monoclonal antibody against CD3. As T cells having CD3 markers play a major role in transplant rejection OKT3 targets T cells and inactivates them facilitating graft acceptance. To immortalize antibody producing B cells. 1×3=3

17. It is the creation of transgenic animals expressing proteins in milk.
    Advantages:
    (i) High production capacity
    (ii) Ease of collection of source material
    (iii) Moderate capital instrument requirements
    (iv) Low operational cost.
    (v) Ease of production and scale up. *(any four)* 1+½×4=3

18. Any 3 of the following- database retrieval tools, blast family, gene level sequences, chromosomal sequences, genome analysis, analysis of gene expression patterns, molecular structure. ½×3 = 1½

Describe their use. ½×3= 1½

19. In a fed batch culture, the fresh medium is continuously fed without removing the growing culture. The volume in the culture vessel increases over a period of time leading to high cell densities. **Benefit**-ideal for intracellular metabolites; differs from batch culture because - batch culture is a closed system and contains limited amounts of nutrients and shows normal growth kinetics, growing cells are exposed to continually changing environment. 1 + 1+1=3

20. Taq polymerase; denaturation, primer annealing and extension; 2^{10} molecules 1+1+1=3

21. Edible parts of plants containing antigens which can serve as vaccines: **Advantages-** no storage problems, easy delivery system and low cost; fruit and other parts that can be eaten raw. 1+1½ + ½ =3

22. Have complex nutritional requirements, grow for limited generations, require carbon dioxide incubators and humidity. pH is monitored using indicators and maintained using buffers; osmolality is monitored using osmometer and maintained with osmolytes such as salts and sugars. 1+½×4 = 3

23. Thermal stability, resistance to denaturation by organic solvents, extreme pH tolerance, improved catalytic efficiency and solubility (any four). Give example of subtilisin wherein MET is changed to ALA to prevent denaturation in presence of bleach. ½×4+1= 3

24. FISH-fluorescent *insitu* hybridization technique involves- introducing fluorescent colours into chromosomes 9 and 22 using nick translation and flourescent nucleotides (red in 9 and green in 22) and then counting the number of yellow cells (ie. having CML) and following their decrease with chemotherapy. 3

25. Institutions where microbial cultures are deposited and maintained; **benefits** (any two)- maintain culture for long period, provide experimental material to investigators, help in protecting intellectual property rights of depositors. MTCC Chandigarh. 1+½×2+½×2 = 3

SECTION D

26 Each protein is identified by its unique peptide map; steps involve- purification→ trypsin treatment → paper electrophoresis → paper chromatography and development of 2-D peptide map.

V.M.Ingram (1957)

Prions are misfolded proteins that can cause diseases. 3+1+1 = 5

27. Based on the expression or non-expression of certain traits such as acquisition of antibiotic resistance or sensitivity to an antibiotic. Blue-white selection method to be explained (insertional inactivation) *E.coli* can be made competent by treating with cold $CaCl_2$ Mandel and Higa 1½+1½+2 = 5

OR

Isolation of DNA fragment (insert) using REs → ligation into vector to generate rDNA → introduction of rDNA into host cell → selection of transformed cells Polylinkers have unique sites for several REs which give flexibility

YAC 3+1½+½ = 5

28. Vector mediated involves the transfer of rDNA through a vector such as the Ti plasmid of *Agrobacterium*. Vectorless involves methods such as chemical mediated, microinjection, electroporation, biolistics

Agrobacterium naturally transfers its genes into plants. 4+1= 5

OR

(i) **Six steps :**

1. Selection of explant
2. Surface sterilization
3. Inoculation onto suitable nutrient medium
4. Growing the culture in the plant tissue culture room
5. Regeneration of plant
6. Acclimatisation

(ii) MS medium

(iii) Type of plant species or plant parts. ½×6+1+1= 5